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The Assessment of Mitochondrial Trna$^{leu(UUR)}$ A3243G Mutation and Their Association with Oxidative Stress among Patients with Inherited type 2 Diabetes Mellitus: A Case Control Study

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KEYWORDS

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Body mass index, Mitochondrial diabetes mellitus, Maternal diabetic inheritance, Reactive oxygen species

ABSTRACT

Introduction/Background: Transfer RNA (tRNA) genes in the mitochondrial DNA genome play an important role in protein synthesis. Insulin secretion does not occur because the mitochondria cannot work optimally. tRNA mutation may also be caused by insulin resistance. In addition, the loss of tRNA modification can cause pancreatic β cell dysfunction. The knowledge about the association of A3243G mitochondrial gene mutation with oxidative/antioxidative systems in the familial T2DM is limited which prompted us to conduct this study.

Objective: To compare the status of mitochondrial gene variant A3243G with oxidative stress markers in familial T2DM as well as in healthy controls, and to find out the association between the parameters, if any

Materials and Methods: A total of 180 subjects (study group: 120; control group: 60) were included in the study. Patients, who attended the Department of Endocrinology and Metabolism, SS Hospital, IMS, BHU, were on diabetes treatment and had a familial history of DM, comprised the study group. Patients underwent clinical, molecular and biochemical assessments. The mtDNA genes were PCR amplified and sequenced. Mitochondrial adenosine triphosphate (ATP) and reactive oxygen species (ROS) were measured

Results: When compared to the control group, greater penetrance was observed among the T2DM patients residing in urban areas ($p = 0.038$). Among the biochemical parameters, no significant difference was found in the level of Hemoglobin (Hb) among both the study groups ($p = > 0.50$), whereas values of FPG and HbA1c were found significantly higher among T2DM subjects compared to control subjects ($p = < 0.001$ and $p = < 0.001$ respectively). Among the oxidative stress markers, the peripheral blood TOS and OSI were found to be significantly higher among T2DM subjects than control subjects ($p = < 0.001$). When compared with the control subjects the peripheral blood TAS level were found to be significantly lower among the T2DM patients ($p = < 0.001$). A positive correlation was found between the FPG and HbA1c levels ($p = < 0.01$) and HbA1c and TOS levels ($p = < 0.01$), whereas a negative correlation was found between TOS and TAS levels ($p = < 0.001$) and HbA1c and TAS levels ($p = < 0.05$) Out of total 120 T2DM patients, three patients were found to carry the A3243G mutation in the mitochondrial

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tRNALeu(UUR) gene in the heteroplasmic form

Conclusion: Antioxidants play an integral role in prevention of metabolic syndrome, DM, and associated complications hence including antioxidant-rich foods in the diet can possibly avoid or atleast delay the occurrence of such complications. The mitochondrial A3243G mutation in the mitochondrial tRNALeu(UUR) gene was found with low frequency but was comparable with previous studies. However screening of large study group with additional screening for mtDNA mutations other than A3243G mutation can further unravel the epidemiological dynamics and prevalence of MIDD or mtDM.

INTRODUCTION

Diabetes mellitus (DM) is one of the most important chronic non-communicable diseases. Over the past few years the continuous upsurge in the incidence of DM and its associated complications has posed a significant burden on healthcare systems worldwide and has emerged as a major public health concern. [1] As per International Diabetes Federation (IDF), 8.8% of the adult population worldwide has diabetes. [2] Current global statistics shows that 463 million individuals have diabetes and the number is estimated to increase to 700 million by 2045, which represents a 51% increase compared to 2019. [2] The prevalence of diabetes in India has risen from 7.1% in 2009 to 8.9% in 2019. [3] India although often referred as diabetes 'capital' of the world ranks second after China in the global diabetes epidemic with 77 million people with diabetes, a number which is estimated to be 134 million by 2045. [2]

Diabetes is a collection of diseases characterized by the presence of chronic hyperglycemia. Pathophysiological mechanisms leading to diabetes can involve an inappropriate secretion of insulin, insulin resistance of the liver, muscle and fat, or combined defects. [4] The risk of an individual to develop diabetes involves a complex interaction between genetic and environmental factors. [4] Gene variants that contribute to the two major forms of diabetes: autoimmune type-1 DM (T1DM) and metabolic syndrome–associated type-2 DM (T2DM), are "low penetrance" variants that modulate the susceptibility of an individual to develop diabetes or not. [5,6] Varieties of gene mutants have been identified over the past few years, and are oftenly referred as "high penetrance" gene variants for diabetes. Carriers of these gene variants have almost 100% chance to develop diabetes at some point during their life span. [4,7] These so called monogenic forms of DM comprise a heterogeneous group of diabetes caused by a single gene defect and the incidence of monogenic DM has increased over the past couple of decades partly due to the greater awareness among the public and partly due to wider availability of genetic testing. [8]

Complex interaction between hereditary and environmental factors leads to progression of diabetes. A family history in this regard is a useful screening tool that can provide valuable genetic information about the probability of developing DM in an individual. [9] It is also important to note here that social and familial set up in India is still rigid and majority of the Indian population still follows the traditional system of consanguineous marriage customs and hence remain endogamous, resulting inheritable genetic diseases. T2DM gets maternally transmitted in many families and has been linked to maternal mitochondrial inheritance. [10,11] As the mitochondrial oxidative phosphorylation (OXPHOS) plays an integral role in glucose stimulated insulin secretion from beta cells, hence mutations in mitochondrial DNA (mtDNA) can lead to DM. [10,11] One of the most commonly reported mtDNA mutation associated with DM is the A3243G mutation in the $tRNA^{leu(UUR)}$ gene which leads to causation of mitochondrial DM (MDM), also called as maternally inherited diabetes and deafness (MIDD) [10-12] and was first described in 1992 by van den Ouveland et al. [12] This diabetogenic and heteroplasmic gene mutation has been is continually recognized among 0.1-1.5% of diabetics and is present on the MTTL1 gene encoding the $tRNA^{Leu(UUR)}$ and is one of the major reason for MIDD. [13] The abnormal glucose metabolism in MIDD is associated with gradual decrease in insulin secretion due to reduced ATP production in pancreatic beta cells with abnormal mitochondria. [14] Considering the implications for treatment and prognosis as well as identification of family members at risk of diabetes, it is imperative to differentially diagnose MIDD from T1DM and T2DM.

Oxidative stress, an unavoidable consequence of living in an oxygen-rich environment, occurs when the synthesis of reactive oxygen and nitrogen species (ROS and RNS) exceeds the capacity of cellular antioxidant defenses to eliminate them. [15] Free radicals and ROS are the primary causes of cellular oxidative damage, a well-known mechanism of cell and tissue damage. Although the low levels of ROS are required for many biochemical processes, but the excessive production

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and/or insufficient removal of ROS can cause oxidative stress, which is defined as an imbalance between the rate at which active oxygen metabolites are formed and the rate at which they are scavenged by enzymatic and non-enzymatic antioxidants. [16,17] The extracellular antioxidant system includes sulphydryl groups (albumin), ascorbate, urate, carotenoids, retinol, α-tocopherol, proteins, and bilirubin, and, has a total antioxidant activity (TAS) that assesses peroxyl-scavenging ability. TAS denotes the antioxidant capacity that remains after ROS have been neutralized. [18]

The knowledge about the association of A3243G mitochondrial gene mutation with oxidative/antioxidative systems in the familial T2DM is limited which prompted us to conduct this study, to compare the status of mitochondrial gene variant A3243G with oxidative stress markers in familial T2DM as well as in healthy controls, and to find out the association between the parameters, if any.

MATERIALS AND METHODS:

Study site and study population: This cross-sectional, case-control study was conducted in Department of Endocrinology and Metabolism, Sir Sundar Lal hospital, Institute of Medical Sciences (IMS), Banaras Hindu University (BHU), Varanasi, India for a period of one year, from November 2016 to October 2017. The study protocol was approved by the Institutional Ethics Committee (IEC).

The study protocol was explained to the participants and prior to their enrollment their written informed consent was obtained. A total of 180 subjects (study group: 120; control group: 60) were included in the study. Patients, who attended the Department of Endocrinology and Metabolism, SS Hospital, IMS, BHU, were on diabetes treatment and had a familial history of DM, comprised the study group. Healthy, non-diabetic and normotensive individuals from the institute staff, who had no evidence of any acute or chronic infection and/or illness were included as control subjects for the study. Patients <18 years of age, without any family history of DM, with chronic diseases and/or mental illness, and those who denied to give written informed consent were all excluded from the study.

Demographic details and the clinical information of the participants were collected at enrollment. Data involving, height, weight, age at diagnosis, glycosylated hemoglobin (HbA1C), fasting plasma glucose (FPG), and family history was collected from office charts, hospital charts, and after a face-to-face interaction with the participants. The familial cases were further categorized into two groups, paternal diabetic inheritance (PDI) and maternal diabetic inheritance (MDI). All the participants consented to biochemical and molecular analysis of their blood samples.

Estimation of body mass index: At the time of hospital admission for treatment, the body mass index (BMI) was determined by using the weight (kg)/height $(m)^2$ formula. The cutoffs for overweight and obesity were taken as 23.0 kg/m2 and 25.0 kg/m2 respectively. [19]

Blood Sample Collection: Blood samples were collected from the study subjects as well as control subjects and were coded to avoid possible bias. A total of 5 ml venous blood was collected from each subject after overnight fasting and was than dispensed into two different vials: 3 ml in clot-activated tubes; 2 ml in ethylene diamine tetra-acetic acid (EDTA) coated vials. The clotted blood was used to extract serum after centrifugation at 3,000 rpm for 10 minutes. Serum samples were collected in micro centrifuge tubes and were utilized for the measurement of TOS and TAS. Remaining Serum samples were stored at -80°C for further investigations. The 2 ml of whole blood (in EDTA vials) was used to extract the genomic DNA.

Isolation of genomic DNA: Fresh blood was used to obtain genomic DNA. The entire experiment was carried out at a temperature of 4°C. The blood samples were homogenized in ice-cold sucrose, EDTA, and Tris-HCl buffer (sucrose 10.8 g, 0.5 mol/l of EDTA: 1 ml, 1 mol/l of Tris-HCl: 2.5 ml, pH 8.0, final volume adjusted to 100 ml by adding nuclease free water; autoclaved and stored at 4°C) and centrifuged at 4000 rpm for 10 min at 4°C to pellet the nuclei and washed once again with sucrose, EDTA, and Tris-HCl buffer. The pure nuclear pellet was suspended in Tris-HCl, EDTA, and NaCl buffer (1 mol/l Tris-Cl: 2.0 ml, 0.5 mol/l EDTA: 1 ml, 5 mol/l NaCl, pH 8.0, final volume adjusted to 100 ml by adding nuclease free water; autoclaved and stored at 4°C) and, sodium dodecyl sulfate (SDS) was added to a final concentration of 1%, which was gently mixed to lyse the nuclei. Proteinase-K (to a final concentration of 100 µg/ml) was added to the lysate and incubated at 37°C overnight. The proteinase-K treated lysate was mixed with an equal volume of Tris saturated phenol (pH 8.0), twice with the phenol: chloroform (1:1) mixture and once with the chloroform isoamyl alcohol (24:1) mixture following centrifugation at 10 000 rpm after each extraction. The final aqueous phase was transferred to a fresh tube. To this aqueous phase, $1/10^{th}$ volume of 3 mol/l of sodium acetate (pH 5.2) and two volumes of ice-cold absolute alcohol were

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added. The DNA was then precipitated, washed with 70% ethanol, air dried, and dissolved in an appropriate volume of TE buffer (pH 8.0). The sample was kept at 37°C till the DNA was completely dissolved.

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP): The $tRNA^{Leu(UUR}$ gene (gene of interest) was PCR amplified using primers described previously by Mkaouar-Rebai et al. [20] with few modifications in the methodology. The primers used to amplify a 528-bp fragment encompassing the full mitochondrial $tRNA^{Leu(UUR}$ gene using PCR were 5'-TCTAGAGTCCATATCAACAA-3' (nt 2953–2972) and 5'-TTTGGTGAAGAGTTTTATGG-3' (nt 3480–3461). PCR was carried out in a 96 well thermal cycler (Veriti, Applied Biosystems), at an annealing temperature of 53°C. Briefly, the 20 µl amplification reaction mixture contained 1x PCR buffer (20 mM Tris-HCl: pH 8.4; 50 mM KCl), 1.5 mM MgCl2, 5 mM dNTP mix, 10 µM of both forward and reverse primers (1 µl each), and 1.25 U Taq polymerase (New England Biolabs, MA, USA) and 2 µl DNA template. The PCR conditions used were initial denaturation at 95°C for 5 min, followed by 30 cycles of 94°C for 30 sec (cyclic denaturation), annealing at 64°C for 30 sec and elongation at 72°C for 60 sec followed by final extension at 72°C for 7 min. 2.0 % agarose gels (BIORON, Ludwigshafen, Germany) in 1xTBE (89 mmol/l of Tris borate and 2 mmol/l of EDTA, pH 8.3) was used to analyze the PCR product.

Following PCR amplification, 23µl of PCR product was digested with 10 U of the restriction endonuclease ApaI (BioLabs) and was than separated on a 2% agarose gel containing ethidium bromide, and was finally visualized under UV transilluminator. PCR product yielded a 528bp fragment in the absence of A3243G mutation (Figure 1a), whereas aforementioned mutation if present, established a restriction at ApaI site, and the digestion yielded two fragments 234bp and 294bp fragments (Figure 1b)

Measurement of Total oxidant status (TOS): The ferrous ion-o-dianisidine complex is oxidized to ferric ion in the serum sample by oxidant molecules, and the reaction is aided by the presence of glycerol molecules in the reaction mixture. The ferric ion and xylenol orange combined to produce a colorful complex in an acidic condition. The color intensity was measured using spectrophotometry, which is proportional to the total amount of oxidants present in the sample. The assay was calibrated using hydrogen peroxide (H2O2), and the result was expressed as micromolar H2O2 equivalent per liter (µmol H2O2 Equivalent/L). [21]

Measurement of Total antioxidant status (TAS): In an acidic medium (the acetate buffer 30mmol/l pH 3.6), the reduced 2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) molecule is oxidized to ABTS+ requiring just H2O2. In an acetate buffer solution, the concentrate ABTS+ particles are more stable over time. The color is slowly and spontaneously faded while a more concentrated solution of acetate buffer as it dilutes at high pH values (the acetate buffer 0.4 mol/l pH 5.8). The number of antioxidants in the sample has an inverse relationship with the rate of bleaching. Trolox, which is widely used as a standard for TAS measurement assays, was utilized to calibrate the reaction. The results were represented in millimoles of Trolox Equivalent per liter (mmol Trolox Equivalent/L). [22]

Calculation of Oxidative stress index (OSI): The OSI value was determined as: OSI (arbitrary unit) = [TOS (µmol H2O2 Eq/L)/TAS (µmol Trolox Eq/L)] ×100. [23]

Statistical analysis: SPSS version 16.0 was used for all statistical analyses (SPSS, Chicago, IL, USA). To compare categorical variables between groups, the Chi-square test was performed. To compare continuous variables between the two groups, the independent sample T-test and Mann Whitney-U tests were used. Multivariate logistic regression and ROC curve analysis were applied to estimate the association and sensitivity of TAS, TOS, and OSI with MDI. A two-sided p-value < 0.05 was considered statistically significant.

RESULTS

Demographic characteristics of the study participants: Table 1 depicts the general demographic characteristics of the study participants. The mean age was compared between both groups and showed no significant difference ($p = 0.909$). The mean BMI for T2DM patients was found significantly higher compared with individuals in the healthy control group ($p = 0.023$). In reference to gender no significant differences were observed between the two groups. When compared to the control group, greater penetrance was observed among the T2DM patients residing in urban areas ($p = 0.038$).

Association of biochemical parameters and oxidative stress markers among the study groups: Association between biochemical parameters and oxidative stress markers among the study groups is represented in Table 2. Among the biochemical parameters, no significant difference was found in the level of Hemoglobin (Hb)

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among both the study groups (p = > 0.50), whereas values of FPG and HbA1c were found significantly higher among T2DM subjects compared to control subjects (p = < 0.001 and p = < 0.001 respectively). Among the oxidative stress markers, the peripheral blood TOS and OSI were found to be significantly higher among T2DM subjects than control subjects (p = < 0.001). When compared with the control subjects the peripheral blood TAS level were found to be significantly lower among the T2DM patients (p = < 0.001).

Some of the important parameters were correlated among the T2DM patients (Table 3) and a positive correlation was found between the FPG and HbA1c levels (p = < 0.01) and HbA1c and TOS levels (p = < 0.01), whereas a negative correlation was found between TOS and TAS levels (p = < 0.001) and HbA1c and TAS levels (p = < 0.05)

Mitochondrial A3243G mutation screening through PCR-RFLP: The A3243G mutation in the mitochondrial $tRNA^{Leu(UUR)}$ gene was screened among the 120 T2DM patients. The A3243G mutation if present resulted in a restriction at ApaI site, and the digestion yielded two fragments: 234bp and 294bp, while in the absence of the aforesaid mutation, a 528 bp fragment corresponding to the PCR amplicon was found. Out of total 120 T2DM patients, three patients were found to carry the A3243G mutation in the mitochondrial $tRNA^{Leu(UUR)}$ gene in the heteroplasmic form (Table 4).

Table 1: Demographic characteristics of the study participants.

Variables		Control Group (n=60)	T2DM Group (n=120)	p value
Age (years)	Mean ± SD	44.10 ± 7.16	44.23 ± 6.73	0.909
BMI	Mean ± SD	23.16 ± 1.50	23.72 ± 1.55	**0.023**
Gender	Male	32 (53.3 %)	58 (48.3 %)	0.635
	Female	28 (46.7 %)	62 (51.7 %)	
Residence	Rural	34 (56.7 %)	47 (39.2 %)	**0.038**
	Urban	26 (43.3 %)	73 (60.8 %)	

BMI: Body mass index; T2DM: Type 2 diabetes mellitus

Table 2: Association between biochemical parameters and oxidative stress markers among the study groups

Variables		Control Group (n=60)	T2DM Group (n=60)	p value
Hb (g/dl)	(mean ± SD)	12.95 ± 1.31	12.81 ± 1.20	0.495
FPG (mmol/L)	(mean ± SD)	92.43 ±9.55	154.30 ± 26.02	**<0.001**
HbA1c (%)	(mean ± SD)	5.46 ± 0.31	7.74 ± 0.54	**<0.001**
TOS (µM)	(mean ± SD)	39.01 ± 15.45	68.56±36.44	**<0.001**
TAS (µM)	(mean ± SD)	756.88 ± 113.50	628.84±111.88	**<0.001**
OSI (AU)	(mean ± SD)	5.28 ± 2.42	11.53 ± 7.60	**<0.001**

AU: Arbitrary Unit; FPG: fasting plasma glucose; Hb: hemoglobin; HbA1c: glycosylated hemoglobin; OSI: oxidative stress index; TAS: total antioxidant status; TOS: total oxidant status; T2DM: type 2 diabetes mellitus

Table 3: Coefficient of correlation between important parameters among the T2DM patients.

Parameters	r value	p value
FPG and HbA1c	0.430**	<0.01
HbA1c and TOS	0.259**	<0.01
TOS and TAS	-0.238**	<0.01
HbA1c and TAS	-0.180*	<0.05

FPG: fasting plasma glucose; HbA1c: glycosylated hemoglobin; TAS: total antioxidant status; TOS: total oxidant status; T2DM: type 2 diabetes mellitus

**p value <0.001 denoted significant level of correlation between the concerned parameters.

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Table 4: Representation of mitochondrial A3243G mutation in diabetic patients with family history

Variables		PDI Group (n=60)	MDI Group (n=60)	p value
A3243G mutation	Yes	1 (1.7 %)	2 (3.3 %)	0.500
	No	59 (98.3 %)	58 (96.7 %)	

PDI: Paternal Diabetic Inheritance, MDI: Maternal Diabetic Inheritance

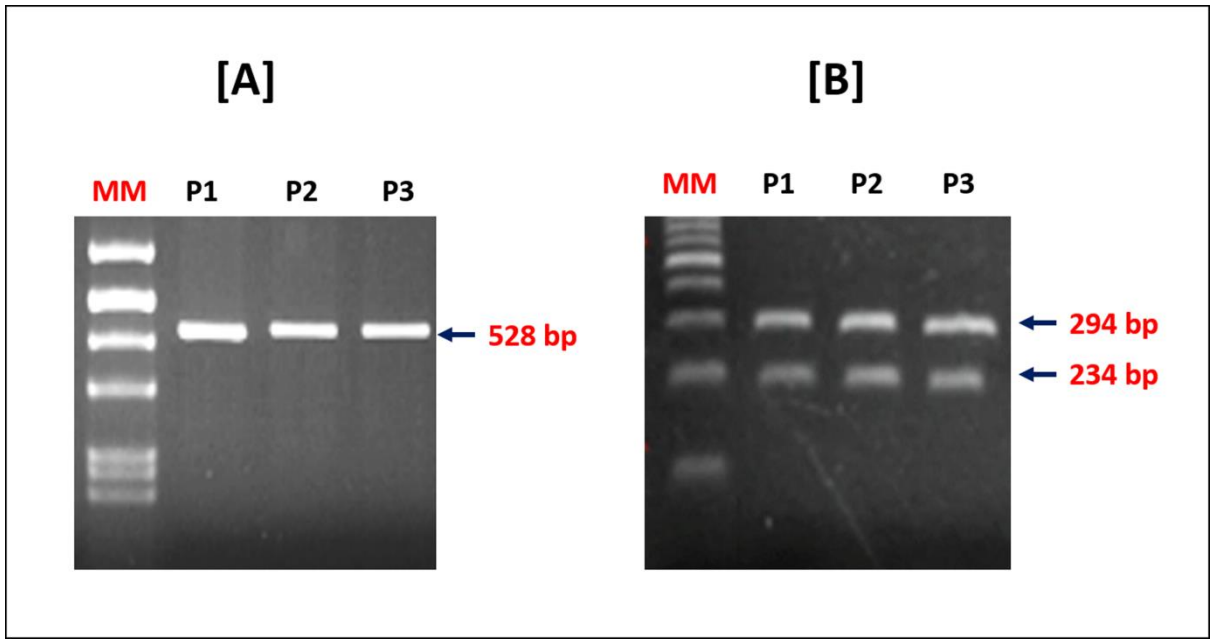

Figure 1: Screening of the A3243G mutation by PCR-RFLP: a 528bp fragment digested with restricted enzyme ApaI.
[A] A3243G mutation absent: PCR product showing undigested 528bp amplicon. MM: 100bp DNA ladder as molecular marker; P1, 2 and 3 are patient samples.
[B] A3243G mutation present: 528bp amplicon cleaved into two fragments of 234bp and 294bp. MM: 100bp DNA ladder as molecular marker; P1, 2 and 3 are patient samples.

DISCUSSION

The present study was carried out on 120 T2DM patients and with age and sex-matched 60 control subjects. Among the T2DM patients, obesity, high BMI, as well as abdominal obesity, were found to be linked with DM, a finding that was consistent with the previous studies. [24-26] Although the BMI of Indians is lower than that of Europeans, but still the risk of diabetes cannot be overlooked with a low BMI. Physical activity has been shown to protect against obesity, cardiovascular disease, and metabolic syndrome, and according to previous studies, sedentary life style coupled with underprivileged physical activity has been linked to diabetes. [24,25]

It was observed in the current study that, patients who resided in urban areas had a greater risk of T2DM. A study by Sadikot et al. also revealed that diabetes prevalence in urban regions was more than two times (5.9%) in comparison to prevalence in rural areas (2.7%), with an overall prevalence of 4.3 percent. [27] A study by Anjana et al. revealed that overall prevalence of diabetes in India was 7.3%, with, Chandigarh having the highest diabetes prevalence of 13.6%. [28] It was also observed that the prevalence was higher: in urban areas than in rural areas; among older age groups and among those with higher socioeconomic status. [28]

As expected, increased HbA1c and FPG levels were noted among the T2DM patients when compared with the control subject, a finding suggestive of excessive glycosylation of hemoglobin and poor diabetes control. Similar findings have been reported previously in studies conducted by various other researchers as well. [29,30] Our study results also demonstrated that FPG and HbA1c were positively correlated with T2DM.

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HbA1c is an important indicator of long-term glycemic control with the ability to reflect the cumulative glycemic history of the preceding two to three months. HbA1c not only provides a reliable measure of chronic hyperglycemia but also correlates well with the risk of long-term diabetes complications. Elevated HbA1c has also been regarded as an independent risk factor for coronary heart disease, stroke and mortality among the diabetic patients. [31] Even an increase of 1% in HbA1c concentration has been found to be associated with about 30% increase in all-cause mortality and 40% increase in cardiovascular or ischemic heart disease mortality, among individuals with diabetes. Whereas reducing the HbA1c level by 0.2% could lower the mortality by 10%. [32] It has been suggested that improving glycemic control in patients with T2DM may be more important than treating dyslipidemia for the prevention of both microvascular and macrovascular complications associated with DM. [33]

We also found a significant rise in TOS and OSI levels, whereas a considerable fall in TAS levels was observed among the T2DM patients and various other researchers have also reported similar findings previously. [34,35] A positive correlation between TOS and HbA1c was noted, whereas negative correlation was found between TAS levels and HbA1c

Unusually elevated levels of free radicals, increased lipid peroxidation and a simultaneous decrease in TAS can lead to increased oxidative stress, which damages the cellular organelles and cell membrane, eventually resulting in development of the complications associated with DM. [36] There is considerable evidence that hyperglycemia represents the main cause of complications of DM, and oxidative stress resulting from increased generation of ROS plays a crucial role in development of such complications. In fact, in the absence of an appropriate response from endogenous antioxidant mechanisms, the redox imbalance causes the activation of stress-sensitive intracellular signaling pathways. The latter play a key role in the development of late complications of DM, as well as in mediating insulin resistance and impaired insulin secretion. Diabetic patients undergo considerable oxidative stress as compared to healthy subjects and the decline in TAS levels might be recognized to enhance oxidative stress. [37] Studies conducted in past have demonstrated (in vitro experiments) that, when compared to a steady high-glucose environment, intermittent high glucose levels can induce ROS overproduction and enhanced cellular apoptosis in human endothelial cells. [38,39]

Mitochondrial diabetes, defined as a mitochondrial disease with chronic hyperglycemia due to inappropriate secretion of insulin, insulin resistance or combined defects, poses a significant challenge for clinicians. It is a rare, monogenic form of diabetes with a frequency of around 1% and accumulating evidences shows that deletions, insertions or point mutations of mtDNA are associated with mitochondrial diabetes. [40] The A-to-G transition at position 3243 of the mtDNA is reported the most prevalent mutation for mitochondrial diabetes worldwide, with a prevalence varying from 0.1% to 10%. [4] The phenotypic expression of this mutation is quite variable, ranging from mild to severe clinical phenotypes. Notably, the A3243G mutation is an important cause of mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS), as well as myoclonic epilepsy and ragged-red fiber disease (MERRF) [Dove [41], and more recently has been recognized as a cause of maternally inherited diabetes and deafness (MIDD). [17]

In the current study, the mitochondrial A3243G mutation was reported in 2.5 % of the diabetic patients. Similar findings have been reported in past by other researchers. [42,43], and a total prevalence of 2.5% of A3243G mutation is usual and patients with early onset diabetes along with maternal familial history are more prone for this otherwise rare monogenic form of diabetes. In contrast to our findings various researchers have reported a low prevalence of A3243G mutation in their studies, ranging from 0.13% to 1.0%. [44,45] A3243G mutation has been detected in a variety of clinical samples including blood, hair follicles, buccal epithelial cells, and muscle biopsy and the quantities of mutant mtDNA A3243G among the diabetic patients are found to be highest in muscle tissue, followed by hair follicles, and lowest in blood cells. [46] Hence it is speculated that, the use of blood samples as the source of mtDNA in the aforementioned studies [44,45], may be the probable reason for detection of mutation in such low frequency. Moreover, as expected the diabetic patients with mitochondrial A3243G mutation in the current study were found to be younger, leaner, had lower blood glucose levels, had neurosensorial deafness and were more frequently related to a maternal history of T2DM when compared to the diabetic patients without any mitochondrial mutation.

In addition to the maternal inheritance and association with progressive neurosensorial deafness, MIDD has following cardinal characteristics: it tends to develop during middle age (25-40 years); to occur in non obese subjects; to frequently require patients to undergo insulin therapy due to progressive insulin secretory defect; to be complicated by other mitochondrial disorders; and to account for 1-2% of the diabetic population. [47] Despite the frequency being relatively low, considering the pharmacotherapeutic and pharmacoeconomic implications, and the associated

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morbidity, early and accurate diagnosis of this rare monogenic mutation seems to be imperative. Although the frequency of A3243G mutation found in the current study was similar to the frequency reported in most of the previous studies but still the other mtDNA mutations have been reported [17], and can possibly play a role in development of MIDD or mtDM. Differential diagnosis of such patients is very important, since these patients could have some severe symptoms, which may be wrongly attributed only to the presence of DM, and the treatment modalities may differ from that of diabetic patients. Detailed prospective studies in this regard need to be conducted to ascertain the prevalence of the same.

CONCLUSION

Estimating plasma antioxidant levels in conjunction with other routine tests may be effective in avoiding the complications associated with DM. Antioxidants play an integral role in prevention of metabolic syndrome, DM, and associated complications hence including antioxidant-rich foods in the diet can possibly avoid or atleast delay the occurrence of such complications. The mitochondrial A3243G mutation in the mitochondrial $tRNA^{Leu(UUR)}$ gene was found with low frequency but was comparable with previous studies. However screening of large study group with additional screening for mtDNA mutations other than A3243G mutation can further unravel the epidemiological dynamics and prevalence of MIDD or mtDM.

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CONFLICTS OF INTEREST

There are no potential conflicts of interest.

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